PATENT

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APPLICANT(S):	Strobel, Gary A., *et al.*
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EXAMINER:	Irene Marx
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DECLARATION UNDER 37 C.F.R. § 1.132

I, Julien Mercier, hereby declare that:

1. I am one of the named inventors of the subject matter claimed in the above-identified patent application.

2. I received a Bachelor of Science degree in Botanical Sciences and a Master of Science degree in Plant Pathology from McGill University in Montreal, Canada and the degree of Doctor of Philosophy in Plant Pathology from Laval University in Quebec, Canada.

3. I have been employed as a Senior Scientist by AgraQuest, Inc. of Davis, California since 2001 and have been and still am engaged in research regarding use of volatile organic compounds that mimic the activity of *Muscodor albus*.

4. After determining that certain volatile organic compounds produced by *Muscodor albus* were able to inhibit the growth of microbes, I conducted experiments to determine how much of each component was needed in various situations to effect inhibition. Determining such ranges

involved straightforward experiments in which various amounts of the volatile organic compounds described in the claims were tested against various microorganisms.

5. Results from experiments conducted after the patent application was filed are described in the attached Exhibits.

6. Exhibit A shows results from experiments conducted in Petri plates against several post-harvest pathogens. These experiments were conducted in the same manner as described in Example 10 of the above-referenced patent application (Example 10), although evaluation of the target organism after exposure to volatile organic compound(s) was accomplished as follows. Instead of evaluating growth of the target organism visually by comparing it to growth of the untreated control, as was done in Example 10, the number of surviving colony forming units in each mycelial plug was determined by dilution plating. In all of the Exhibits, results reported as log CFU/plug refer to surviving colony forming units, after exposure to volatile organic compound(s). Reduction by one log indicates that 90% of the target organism colony forming units (CFU) were killed.

7. Exhibit B shows results from experiments conducted on Petri plates containing a fungal or bacterial suspension applied to culture medium. The pathogen-containing Petri plates and the volatile organic compound(s) were placed in 6-L fumigation boxes equipped with a fan. The Exhibit provides a short summary of the experimental protocol, which is similar to the protocol described in Example 10, except in this case the volume of the box, rather than of a sealed Petri plate, was used to expose the target organisms to the volatile organic compound(s). The target organisms were post-harvest pathogens (*Penicillium expansum*, *Geotrichum citri-aurantii*, *Aspergillus niger*, and *Erwinia carotorova*). *Penicillium expansum* and *Aspergillus niger* are also components of toxic mold that infest buildings. These experiments were conducted during May through June 2006.

8. Exhibit C shows results from experiments conducted against *Penicillium expansum* and *Rhizoctonia solani* over a period of time spanning June 2005 through April 2006. Some experiments were conducted in 35-L fumigation boxes, with a fan, and others were conducted in 6-L fumigation boxes, with a fan. Experiments were conducted in the same manner as described in Exhibit B, with Petri plates containing a fungal suspension placed in the boxes along with the volatile organic compound(s). As in Exhibit B, in Table A of Exhibit C, results were evaluated

visually as a percentage of untreated control. In contrast, in Tables B-F, the number of surviving colony forming units in each mycelial plug were determined by dilution plating, as in the experiments described in Exhibit A.

I, being warned that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and may jeopardize the validity of the application or any patent issuing thereon, declare that all statements made of the my own knowledge are true and that all statements made on information and belief are believed to be true.

Dated: 4 July 2006 By: Julien Mercier

Julien Mercier, Ph.D.

Exhibit A

* In the experiments described below, the pathogen was grown until sporulation occurred. Then, plugs were cut out and added to a Petri plate, along with the volatile organic compounds described in the tables below. The head space in the sealed Petri plates was 65 mL. The number of surviving colony forming units (CFU) in each mycelial plug was determined by dilution plating.

Tests against various organisms using isobutyric acid or 2-methyl-1-butanol

Isobutyric acid

Log CFU/plug ± std dev.

Pathogen	Control	10 µl	20µl	50 µl	100 µl	Date tested
Geotrichum citri-aurantii	6.861 ± 0.018	3.113 ± 0.034	0	0	0	10/17/2003
Penicillium digitatum	7.335 ± 0.023	0	0	0	0	10/22/2003
Penicillium expansum	7.598 ± 0.028	3.620 ± 0.183	2.608 ± 0.146	2.58 ± 0.144	2.439 ± 0.414	8/25/2003

2-methyl-1-butanol

Log CFU/plug ± std dev.

Pathogen	Control	10 µl	20µl	50 µl	100 µl	Date tested
Geotrichum citri-aurantii	6.822 ± 0.049	2.250 ± 0.575	0	0	0	10/3/2003
Penicillium digitatum	7.212 ± 0.040	0	0	0	0	10/29/2003
Penicillium expansum	tmc	7.601 ± 0.109	3.545 ± 0.163	3.365 ± 0.241	0	8/25/2003

* tmc = too many too count

Tests against *Penicillium expansum* with various mixtures of volatile organic compounds

Test 1	date: 1/6/04		
2-methyl-1-butanol (µl)	0	60	20
Ethyl butyrate	0	60	80
Log CFU/plug ± std dev.	7.064 ± 0.058	6.744 ± 0.071	6.7678 ± 0.037

Test 2	date: 1/6/04		
2-methyl-1-butanol (µl)	0	60	20
Ethyl propionate	0	60	80
Log CFU/plug ± std dev.	7.064 ± 0.058	6.740 ± 0.014	6.736 ± 0.079

Test 3	date: 2/2/04		
2-methyl-1-butanol (µl)	0	20	20
Methyl-2-methyl butyrate	0	0	50
Log CFU/plug ± std dev.	8.321 ± 0.059	6.853 ± 0.028	6.874 ± 0.078

Test 4	date: 2/2/04		
2-methyl-1-butanol (µl)	0	20	20
Phenethyl alcohol	0	0	50
Log CFU/plug ± std dev.	8.3205 ± 0.059	6.853 ± 0.028	7.116±0.170

Test 5	date: 2/27/04					
2-methyl-1-butanol (µl)	0	20	20	20	0	0
methyl isobutyrate (µl)	0	0	50	0	50	0
ethyl isobutyrate (µl)	0	0	0	50	0	50
Log CFU/plug ± std dev.	7.559 ± 0.084	7.117 ± 0.101	7.483 ± 0.149	7.175 ± 0.029	7.441 ± 0.032	7.512 ± 0.057

Test 6	date: 3/12/04						
Isobutyric acid (µl)	0	10	10	10	0	0	10
Phenethyl alcohol (µl)	0	50	0	0	50	0	50
Isobutyl alcohol	0	0	50	0	0	50	50
Log CFU/plug ± std dev.	7.667 ± 0.045	7.368 ± 0.084	0	6.220 ± 0.053	7.522 ± 0.041	7.286 ± 0.013	4.461 ± 0.142

Test 7	date: 3/22/04						
Isobutyric acid (µl)	0	10	10	10	0	0	10
Methyl-2-methylbutyrate	0	50	0	0	50	0	50
Methyl isobutyrate	0	0	50	0	0	50	50
Log CFU/plug ± std dev.	8.212 ± 0.04	7.075 ± 0.097	6.518 ± 0.078	0	7.535 ± 0.028	7.517 ± 0.034	7.203 ± 0.034

Test 8	date: 3/29/04							
Isobutyric acid (µl)	0	10	10	10	10	0	0	0
Ethyl propionate	0	50	0	0	0	0	50	0
Ethyl isobutyrate	0	0	50	0	0	50	0	0
Ethyl butyrate	0	0	0	50	0	0	0	50
Log CFU/plug ± std dev.	7.689 ± 0.044	7.263 ± 0.014	6.379 ± 0.04	7.111 ± 0.06	6.185 ± 0.111	7.457± 0.023	7.441 ± 0.018	7.380 ± 0.018

Test 9	date:4/2/04							
2-methyl-1-butanol (µl)	0	20	20	0	0	0	0	20
Isobutyl alcohol	0	50	0	50	0	0	0	0
Isobutyric acid	0	0	0	0	10	10	0	10
Methyl isobutyrate	0	0	0	0	50	0	50	0
Log CFU/plug ± std dev.	7.854±0.04	0	6.486 ± 0.162	4.830 ± 0.03	6.414± 0.38	5.885 ± 0.06	7.453 ± 0.076	5.100 ± 0.05

Tests against *Penicillium expansum* with various combinations of isobutyric acid, 2-methyl-1-butanol and isobutyl alcohol

Test A	date: 3/4/04					
Isobutyric acid	0	15 µl	10 µl	7.5 µl	5 µl	2.5 µl
Log CFU/plug ± std dev.	8.419 ± 0.035	0	5.707 ± 0.03	7.452 ± 0.023	7.349 ± 0.087	7.419 ± 0.035

Test B	date: 2/2/04		
2-methyl-1-butanol (µl)	0	20	20
isobutyl alcohol (µl)	0	0	50
Log CFU/plug ± std dev.	8.321 ± 0.059	6.853	0

Test C	date: 2/18/04				
2-methyl-1-butanol (µl)	0	20	0	20	20
Isobutyl alcohol (µl)	0	0	20	20	50
Log CFU/plug ± std dev.	7.619 ± 0.031	7.263 ± 0.103	7.534 ± 0.052	5.357 ± 0.117	4.844 ± 0.042

Test D	date: 4/14/04								
2-methyl-1-butanol (µl)	0	20	20	20	20	20	20	0	0
Isobutyric acid	0	10	10	10	10	10	0	0	10
Isobutyl alcohol	0	0	5	10	20	40	0	20	0
Log CFU/plug ± std dev.	7.902 ± 0.05	4.830 ± 0.06	4.906 ± 0.02	0	0	0	5.040 ± 0.03	7.091 ± 0.02	6.165 ± 0.03

Test E	date: 4/20/04								
Isobutyric acid (µl)	0	10	10	10	10	10	0	0	5
Isobutyl alcohol	0	10	10	10	10	0	10	0	5
2-methyl-1-butanol	0	5	10	15	20	0	0	10	10
Log CFU/plug ± std dev.	7.914 ± 0.08	0	0	0	0	6.078 ± 0.04	7.436 ± 0.02	7.599 ± 0.06	5.810 ± 0.04

Test F	date: 4/29/04					
Isobutyric acid (µl)	0	5	5	10	10	5
Isobutyl alcohol	0	10	5	5	0	0
2-methyl-1-butanol	0	10	5	5	0	0
Log CFU/plug ± std dev.	7.940 ± 0.03	0	0	0	0	7.440 ± 0.047

Test G	date: 5/6/04					
Isobutyric acid (µl)	0	5	5	2.5	5	0
Isobutyl alcohol	0	10	5	2.5	0	5
2-methyl-1-butanol	0	10	5	2.5	0	5
Log CFU/plug ± std dev.	7.902 ± 0.027	5.982 ± 0.00	0	0	7.227 ± 0.07	7.531 ± 0.033

Test H	date: 5/6/04					
Isobutyric acid (µl)	0	5	5	2.5	5	0
Isobutyl alcohol	0	10	5	2.5	0	5
2-methyl-1-butanol	0	10	5	2.5	0	5
Log CFU/plug ± std dev.	7.917±0.03	5.836±0.135	0	3.541±0.10	7.425±0.04	7.514±0.021

Exhibit B

1. All fumigation experiments done in 6-L fumigation boxes with fan.
2. Isobutyric acid (IBA) in various amounts was placed in box along with test organism.
3. IBA was volatilized with the aid of a fan and organism exposed to volatile for 16-18 h at room temperature.
4. Growth was evaluated visually as a percentage of the untreated control which was set to 100%.

A. Experiments against *Penicillium expansum* on potato dextrose agar plates

Amount of IBA used in μL	% growth (expt 1)	% growth (expt 2)
Control	100	100
5	100	100
10	90	90
15	80	80
25	60	70
35	15	20
45	5	5
55	0	0
75	0	0
95	0	0
125	0	0
150	0	0

B. Experiments against *Geotrichum citri-aurantii* on potato dextrose agar plates

Amount used in μL	% growth (expt 1)	% growth (expt 2)
Control	100	100
5	100	100
10	100	100
15	100	100
25	100	100
35	90	75
45	75	60
55	40	20
75	5	5
95	0	0
125	0	0
150	0	0

C. Experiments against *Aspergillus niger* on potato dextrose agar plates

Amount used in µL	% growth (expt 1)	% growth (expt 2)
Control	100	100
5	100	100
10	100	90
15	90	90
25	80	90
35	50	40
45	40	40
55	5	8
75	0	0
95	0	0
125	0	0
150	0	0

D. Experiments against the bacterium *Erwinia carotorova* on nutrient agar plates

Amount used in µL	% growth (expt 1)	% growth (expt 2)
Control	100	100
5	60	30
10	0	0
15	0	0
25	0	0
35	0	0
45	0	0
55	0	0
75	0	0
95	0	0
125	0	0
150	0	0

Exhibit C

A. Two-compound and three-compound mixture experiments with isobutyric acid (IBA), methyl isobutyrate and 2-methyl-1-butanol tested in a 35-L fumigation box with fan using *Penicillium expansum* (Penex) and *Rhizoctonia solani* (Rhizoc) grown on replicate plates. For Penex, growth was evaluated visually as a percentage of untreated control, which was set at 100%. For Rhizoc, growth was evaluated visually in terms of colony diameter. Results are provided for each plate, as X/X.

IBA added (µL)	Methyl isobutyrate added (µl)	2-methyl-1-butanol added (µL)	Exposure time	Penex % growth	Rhizoc colony diameter (mm)
Control			24 h	100	80
			48 h	100	80
96	3.0	0	24 h	80/70	0
			48 h	80/70	0
58	15	0	24 h	80/70	0
			48 h	80/70	0
96	0	3.2	24 h	80/80	0/10
			48 h	70/70	8/9
96	0	6.4	24 h	80/80	8/9
			48 h	80/80	10/11
96	1.5	1.7	24 h	80/80	10/8
			48 h	60/60	9/8
110	0	0	24 h	60/60	0
			48 h	80/80	0
200	0	0	24 h	10/1 (0/0 on 2nd expt)	
			48 h	10/30	
200	2.28	0	24 h	10/7	
			48 h	10/7	
200	6.84	0	24 h	15/5	
			48 h	5/5	

* Table continued from page 1.

IBA added (µL)	Methyl isobutyrate added (µl)	2-methyl-1-butanol added (µL)	Exposure time	Penex % growth	Rhizoc colony diameter (mm)
200	22.8	0	24 h	15/2	
			48 h	20/5	
200	0	2.93	24 h	1/1	
			48 h	2/2	
200	0	8.8	24 h	1/1	
			48 h	2/5	
200	0	29.3	24 h	0/2	
			48 h	1/5	
250	0	0	24 h	0/0	
160	0	2.93	24 h	40/30	
			48 h	30/10	
160	0	8.8	24 h	25/25	
			48 h	20/20	
160	0	29	24 h	25/25	
			48 h	20/25	

B. IBA tested in 6-L fumigation box with fan using plugs of *Penicillium expansum* (Penex) and *Cladosporium cladosporioides* (Clah) exposed for 16-18 h at room temp. CFU in the following tables refers to colony forming units.

IBA added (µL)	Log CFU/plug (± SD) Penex	Log CFU/plug (± SD) Clah
Control	7.66 (0.055)	6.94 (0.06)
10	7.22 (0.418)	0
25	7.65 (0.078)	0
40	7.35 (0.108)	0
50	6.86 (0.338)	0
60	1.93 (1.67)	0
75	2.89 (0.111)	0

C. Combination of IBA and isobutyl alcohol tested in 6-L fumigation box with fan using plugs of *Penicillium expansum* (Penex) exposed for 16-18 h at room temp.

IBA added (µL)	Isobutyl alcohol added (µl)	Log CFU/plug (± SD) Penex
Control		7.57 (0.03)
50	0	0.77 (1.33)
50	5	4.60 (0.78)
50	10	5.84 (0.48)
50	20	4.54 (0.54)
50	30	2.40 (0.17)
50	40	0

D. Combination of IBA and 2-methyl-1-butanol tested in 6-L fumigation box with fan using plugs of *Penicillium expansum* (Penex) exposed for 16-18 h at room temp.

IBA added (µL)	2-methyl-1-butanol added (µL)	Log CFU/plug (± SD) Penex
Control		7.61 (0.04)
50	0	0
50	5	0
50	10	0
50	20	0
50	30	0
50	40	0

E. Combination of IBA and isobutyl alcohol tested in 6-L fumigation box with fan using plugs of *Penicillium expansum* (Penex) exposed for 16-18 h at room temp.

IBA added (µL)	Isobutyl alcohol added (µl)	Log CFU/plug (± SD) Penex
Control		7.79 (0.16)
35	0	0
35	2	4.62 (0.21)
35	5	4.99 (0.32)
35	10	4.85 (0.61)
35	20	0
35	30	0

F. Combination of IBA and isobutyl alcohol tested in 6-L fumigation box with fan using plugs of *Penicillium expansum* (Penex) exposed for 16-18 h at room temp.

IBA added (µL)	Isobutyl alcohol added (µl)	Log CFU/plug (± SD) Penex
Control		7.66 (0.11)
15	0	1.03 (1.78)
25	0	6.89 (0.07)
25	2	7.10 (0.10)
25	5	6.94 (0.14)
25	10	0
25	20	2.62 (0.28)

Several rusts have been prepared for an extended long-term viability study, namely Melampsora medusae Thüm ATCC PR 70 (over-wintered telia on Populus deltoides Marsh., April 1971), Gymnosporangium clavipes (Cke. & Pk.) Cke. & Pk. ATCC PR-71 (telia on Juniperus virginiana, April 1971), G. nidus-avis Thaxt. ATCC CPR-69-3 (telia on J. virginiana, April 1969), G. juniperi-virginianae Schw. ATCC CPR 69-8 (telia on J. virginiana, April 1969), Puccinia windsoriae Schw. ATCC CPR 69-14 (over-wintered telia on Tridens flavus, April 1969), and P. helianthi Schw. ATCC PR-74 (urediospores from Helianthus annuus, April 1972). Other species may be added as material becomes available. The plan is to test viability annually the first 3 years and then at 5-year intervals for as long as the original material lasts. There are between 40 and 120 ampules of each of these particular strains. The procedures for continuation of this study are on file at ATCC for reference by future generations of workers.

Literature Cited

1. BROMFIELD, K. R. 1964. Cold-induced dormancy and its reversal in uredospores of Puccinia graminis var. tritici. Phytopathology 54: 68-74.
2. KILPATRICK, R. A., D. L. HARMON, W. Q. LOEGERING, and W. A. CLARK. 1971. Viability of uredospores of Puccinia graminis f. sp. tritici stored in liquid nitrogen, 1960-1970. Plant Dis. Reptr. 55: 871-873.
3. LOEGERING, W. Q., H. H. McKINNEY, D. L. HARMON, and W. A. CLARK. 1961. A long term experiment for preservation of uredospores of Puccinia graminis tritici in liquid nitrogen. Plant Dis. Reptr. 45: 384-385.

CONTROL OF POSTHARVEST DECAY OF FRESH RASPBERRIES BY ACETALDEHYDE VAPOR

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Scientific Publication No. A1902, Contribution No. 4820, University of Maryland Agricultural Experiment Station, College Park.

ABSTRACT

Acetaldehyde vapor, known to occur in mature fruits, was evaluated for control of postharvest decay of fresh raspberries. Decay of raspberries, inoculated with Botrytis cinerea, was controlled by fumigation with acetaldehyde vapor. Treatments with 0.25% or 0.5% acetaldehyde vapor for 70 min were as effective as those with 1% and greater concentrations. Acetaldehyde vapor at these concentrations inhibited germination of B. cinerea. Objectionable off-flavor was not detected in berries exposed to 0.5% or lower concentrations of acetaldehyde vapor.

Plant Dis. Reptr. 57: 795-797.

Storage and marketing life of fresh raspberries (Rubus strigosus) is severely reduced by postharvest decay. Decay losses of raspberries during transit and unloading have been estimated around five million dollars annually (11). A major portion of these losses have been attributed to Botrytis cinerea Pers. ex Fr. Several postharvest decay control measures have been suggested with limited commercial application (3).

Acetaldehyde (Aa) is one of the natural volatile compounds and imparts flavor to fruits and vegetables (4, 6, 7). The fungicidal activity of Aa vapor has been demonstrated by research workers (1, 2, 5, 8). Acetaldehyde was the main carbonyl compound present in volatile fractions of Trichoderma viride isolates and accounted for vapor phase sporostasis of several fungi (2).

Acetaldehyde vapor caused slight growth inhibition of Fomes annosus at 100 ppm and complete inhibition at 300 ppm and above. The antagonistic properties of Trichoderma isolates were attributed to volatile production and acetaldehyde vapor was one of the inhibitory metabolites.

This investigation was undertaken to determine the effectiveness of Aa vapor in reducing B. cinerea decay of fresh raspberries, and organolyptic detection of objectionable off-flavor in berries exposed to Aa vapor.

MATERIALS AND METHODS

Raspberries were obtained from Plant Research Farm, University of Maryland, College Park. Botrytis cinerea was isolated from strawberries and single spore cultures were grown on pea agar (PA) at 24° C for 10 days. Spores were collected by flushing culture plates with sterile distilled water containing one drop per liter of polyoxyethylene monolaurate to aid spore dispersal. Spore concentration of 2.3×10^6/ml was used for inoculation.

Conidia were sprayed on 50 g of raspberries which were then exposed to Aa vapor in a fumigation chamber. Similar uninoculated raspberries were used as control. The concentrations of Aa vapor, expressed as percentage of atmosphere by volume and exposure times (min) were 0.25% for 70 min, 0.5% for 70 min, 1% for 60 min and 2% for 30 min. Fumigated raspberries were incubated for 72 hr at 24° C and 75% relative humidity. Numbers of decayed berries were counted after incubation. The experiment was replicated four times.

Flavor evaluation of treated berries was conducted by five judges. The unexposed berries were used as known and unknown standards to reduce error in flavor ratings. Numerical flavor ratings of 5 (better than standard), 4 (equal to standard), 3 (below standard but no off-flavor), 2 (below standard and definite off-flavor) and 1 (unacceptable) were assigned to the judgments and mean scores were calculated for each treatment.

Table 1. Effect of acetaldehyde vapor on decay of fresh raspberries inoculated with Botrytis cinerea[a].

Acetaldehyde		Percentage[b]
Percentage	Exposure (min)	decay
0	0	100
0.25	70	0
0.50	70	0
1.00	60	0
2.00	30	0

[a]Incubated for 72 hr at 55°C and 75% relative humidity after exposure to acetaldehyde (Aa).
[b]Percentage calculated from mean of four replications.

Table 2. Effect of acetaldehyde vapor on flavor of raspberry[a].

Acetaldehyde treatment		Mean Flavor Panel Ratings[b]	
		Standard	
Concentration (%)	Exposure (Min)	Known	Unknown
0	0	4.0 b	2.7 b
0.25	70	1.0 b	3.7 a
0.5	70	4.7 a	2.7 b
1.0	60	2.7 c	2.3 c
2.0	30	2.0 d	2.3 c

[a]Incubated for 72 hr at 55°C and 75% relative humidity after exposure to Aa. [b]Numerical flavor ratings of 5 (better than standard), 4 (equal to standard), 3 (below standard, but no off-flavor), 2 (below standard and definite off-flavor) and 1 (unacceptable) were assigned to judgments and mean flavor ratings were calculated for each treatment. Means followed by same letter in a column were not [illegible] at 5% level (P = 0.05).

RESULTS

Decay control: Raspberries inoculated with B. cinerea decayed within 72 hr when not exposed to Aa vapor, but the decay was prevented by exposing the berries to various Aa vapor concentrations (Table 1). At low concentrations of Aa vapor, longer exposure was required for control.

Organolyptic evaluation: Flavor of raspberries exposed to 0.25% Aa vapor for 70 min was preferred in comparison with unknown standard (Table 2). Berries exposed to 0.5% Aa vapor for 70 min had the same panel preference as that of the unknown standard. Flavor of berries exposed to 0.25% Aa vapor, however, had the same panel preference when compared with a known standard although berries exposed to 0.5% for 70 min were preferred. Objectionable off-flavor was detected at 1% Aa vapor and above.

DISCUSSION

Decay of fresh raspberries, inoculated with B. cinerea, can be controlled by various Aa vapor treatments. Fumigation with Aa vapor at 0.25% or above prevented decay development of the raspberries. The effectiveness of Aa vapor treatment was a factor of concentration and exposure time. Several fruits have been successfully stored free of storage decay in atmosphere containing low Aa vapor concentrations (8, 9, 10).

Acetaldehyde vapor was lethal to the germinating spores of B. cinerea at concentrations that effectively prevented decay development. Botrytis cinerea could not be reisolated from infected berries after exposure to Aa vapor. In the absence of fumigation, however, the pathogen was readily isolated from the infected tissues. Decay control of raspberries inoculated with B. cinerea is probably mediated by contact vapor toxicity of Aa.

The absence of objectionable off-flavor in berries treated with 0.5% or lower concentrations of Aa vapor suggests the potential value of this fumigant in preventing decay losses of raspberries during storage.

Literature Cited

1. AHARONI, Y., and G. J. STADELBACHER. 1973. The toxicity of acetaldehyde vapors to postharvest pathogens of fruits and vegetables. Phytopathology 63: 544-545.
2. DENNIS, C., and J. WEBSTER. 1971. Antagonistic properties of species-groups of Trichoderma. II. Production of volatile antibiotics. Trans. Brit. Mycol. Soc. 57(1): 41-48.
3. ECKERT, J. W., and N. F. SOMMER. 1967. Control of diseases of fruits and vegetables by postharvest treatments. Ann. Rev. Phytopath. 5: 391-432.
4. FIDLER, J. C. 1968. The metabolism of acetaldehyde by plant tissues. J. Exp. Botany 19: 41-51.
5. FRENCH, R. C. 1962. Interaction of aldehydes with uredospores of stem rust of wheat. Botan. Gaz. 124: 121-128.
6. HAYES, E. R. 1963. Acetaldehyde. In A. Standen (Exec. Ed.), Encyclopedia of Chemical Technology. Kirk-Othmer. pp. 77-95.
7. NURSTEN, H. E. 1970. Volatile compounds: the aroma of fruits. In A. C. Hulme (Ed.), The Biochemistry of Fruits and Their Products. Academic Press, London & New York. Vol. 1. pp. 239-268.
8. SHAW, G. W. 1969. The effect of control atmosphere storage on the quality and shelf life of fresh strawberries with special reference to Botrytis cinerea and Rhizopus nigricans. Ph. D. Dissertation, Univ. of Maryland, College Park. 62 pp.
9. STADELBACHER, G. J., and Y. AHARONI. 1971. Acetaldehyde vapor treatment to control postharvest decay in strawberry. Hort. Sci. 6(3): 20.
10. TOMPKINS, R. G., and S. A. TROUT. 1931. The prevention of decay of stored fruit by the use of volatile compounds. Gt. Brit. Dept. Sci. Ind. Res., Food Invest. Board Rept. pp. 117-119.
11. UNITED STATES DEPARTMENT OF AGRICULTURE. 1965. Losses in Agriculture. Agriculture Handbook 291: 1-120.

Fumigation with acetic acid vapor to control decay of stored apples

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Fumigation with acetic acid vapor to control decay of stored apples.

Abstract — Introduction. Apples are potentially subject to blue mold decay caused by *Penicillium expansum* if stored at 1 °C for three or more months or if wounded during handling. Results from trials with apples contaminated with conidia of *P. expansum* and fumigated in small chambers with acetic acid (AA) vapor indicated that fruit could be sterilized to reduce decay without effect on fruit quality. The objective of this study was to determine if larger quantities of apples treated with AA vapor would have less decay after storage and/or wounding. It was also important to determine if fumigation would affect apple quality and aroma. **Materials and methods**. Apple cultivars were harvested at commercial maturity for use in AA fumigation trials. Apples artificially or naturally contaminated with conidia of *P. expansum* were fumigated with AA vapor in a 1 m^3 gas tight chamber at 10 °C for 1 h to 24 h or dipped in 450 µg thiabendazole × L^{-1} solution. Fruit fumigated in standard wooden or plastic apple boxes, or small wooden bins were either wounded and evaluated for decay after a week at 20 °C or stored at 1 °C for three or more months and evaluated for decay. Then apple quality was assessed. **Results**. Apples naturally contaminated with *Penicillium* spp. that had been stored at 1 °C in air storage and treated with AA vapour had 50% less decay than the control fruit. In another experiment, AA fumigation was as effective as thiabendazole in reducing decay. AA fumigation reduced decay of fruit coming out of storage for apples stored for 3 months, and a second AA fumigation reduced infection of wounds on these same apples. AA fumigation before storage did not affect apple quality or vinegar aroma. **Discussion**. AA fumigation showed great potential for reducing decay in stored apples. It could be used as an organic alternative to synthetic fungicides for control of blue mold decay.

Canada / *Malus* (fruits) / postharvest decay / fungal diseases / *Penicillium expansum* / disease control / control methods / fumigation / acetic acid / thiabendazole / quality

Contrôle de la détérioration des pommes stockées par traitement à la vapeur d'acide acétique.

Résumé — Introduction. Les pommes sont potentiellement sujettes à la moisissure bleue provoquée par *Penicillium expansum* si elles sont stockées à 1 °C pendant 3 mois ou plus, ou si elles sont blessées pendant la manutention. Les résultats d'essais effectués avec des pommes contaminées par des conidies de *P. expansum* et fumigées dans un local restreint avec de la vapeur d'acide acétique (AA) ont montré que les fruits stérilisés pouvaient être moins détériorés, sans que leur qualité ne soit altérée. L'objectif de cette étude a été de déterminer s'il était possible de protéger de plus grandes quantités de pommes contre ces détériorations après stockage et/ou après blessures en les traitant à la vapeur de AA. Il s'agissait également de déterminer si la fumigation affectait la qualité et l'arôme des pommes traitées. **Matériel et méthodes**. Des pommes de différents cultivars ont été récoltées au stade de maturité commerciale puis elles ont été artificiellement ou naturellement contaminées avec des conidies de *P. expansum*. Elles ont été alors fumigées avec de la vapeur de AA dans une une chambre de 1 m^3, étanche au gaz, à 10 °C pendant 1 h à 24 h ou plongées dans une solution à 450 µg thiabendazole × L^{-1}. Les fruits fumigés ont été soit blessés puis évalués vis-à-vis de leur détérioration après 1 semaine à 20 °C, soit stockés à 1 °C pendant 3 mois ou plus puis évalués quant à leur état. La qualité des pommes a alors été estimée. **Résultats**. Les pommes naturellement contaminées avec *Penicillium* spp., stockées à l'air à 1 °C et traitées à la vapeur de AA ont été moitié moins détériorées que les fruits témoins. Dans une autre expérimentation, la fumigation avec AA a été aussi efficace que le thiabendazole pour réduire la moisissure. La fumigation avec AA a réduit la détérioration des fruits après stockage pendant 3 mois, et un deuxième traitement à AA a diminué l'infection des blessures sur ces mêmes pommes. La fumigation avec AA effectuée avant stockage n'a pas affecté la qualité des pommes ni leur arôme. **Discussion**. Les fumigations à la vapeur de AA sont donc potentiellement très intéressantes pour réduire la détérioration des pommes stockées. Elles pourraient remplacer les fongicides synthétiques pour le contrôle de la moisissure bleue de la pomme.

Canada / *Malus* (fruits) /maladie post récolte /maladie fongique / *Penicillium expansum* / contrôle de maladies / méthode de lutte / fumigation / acide acétique / thiabendazole / qualité

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1. Introduction

The Okanagan and associated valleys within British Columbia, Canada, produce approximately 50% of the Canadian apple crop of which approximately one half is stored for three or more months. Fruit stored in air or controlled atmosphere (CA) storage at temperatures at or near 0 °C are subject to decay by fungi such as *Penicillium expansum* and *Botrytis cinerea* [1, 2]. The use of synthetic fungicides is frowned upon because of the concern that they are carcinogenic and harmful to the environment. Packinghouse managers would rather not use fungicides because residues on the fruit could be associated with health hazards such as cancer [3]. Furthermore, organic growers are not allowed to use synthetic fungicides and growers who produce apples for baby food processors must rely on preharvest decay control strategies.

Penicillium expansum, also known as blue mold, is the most important causal agent of apple decay in Canada [4] and the United States [5]. Control of blue mold relies on the integration of several practices including selection of apple cultivars resistant to decay, harvesting fruit with optimal maturity, handling to avoid bruising and injury, bin and packinghouse sanitation, and use of fungicides or biologicals to control blue mold. In Canada and the United States, the only fungicide acceptable by the fruit industry for postharvest use on apples is thiabendazole although *P. expansum* has developed resistance to it [6]. For the above reasons, an acceptable alternative to thiabendazole is required for control of decay in stored apples.

Alternatives to synthetic fungicides that need further study as possible treatments to control postharvest decay are fumigants. Fumigation is not widely used as a method of controlling postharvest decay although fumigants have some particularly useful properties. Fumigants can diffuse through space and penetrate into protected places that are inaccessible to liquid or solid pesticides, and exert their effect during the exposure period but diffuse away afterwards leaving little or no residue [7]. The only commercial crop fumigated to prevent decay are table grapes. Sulfur dioxide fumigation is primarily used to prevent the contact spread of *Botrytis cinerea* on table grapes during storage [8].

Use of acetaldehyde vapor to control postharvest pathogens has been investigated in apples [9], cherries [10] and oranges [11]. Acetaldehyde was effective but phytotoxic on cherries and oranges. Stadelbacher and Prasad [9] showed that decay of apples inoculated with *P. expansum* was prevented by acetaldehyde fumigation at concentrations as low as 0.5% (v/v) for 180 min, or 3% for 30 min. As far as we are aware, this treatment has not been tried in commercial storages probably because of concerns with flammability and carcinogenicity. On the other hand, acetic acid (AA) in air at low concentrations is not flammable, explosive, or carcinogenic [12] and is very effective against molds such as *P. expansum* that cause storage decay [13]. Subsequent studies showed that AA fumigation would control postharvest decay caused by other pathogens such as *Monilinia fructicola* and *Botrytis cinerea* on apricots [14], cherries [15, 16], citrus [16], grapes [17], peaches [14], nectarines [14] and strawberries [18]. Acetic acid vapor treatment for the control of postharvest decay has been reviewed [19] identifying additional uses in food processing and modified atmosphere packaging.

The objective of this study was to determine if relatively large quantities of apples treated with acetic acid vapor would have less decay after storage and/or after wounding. Fumigation parameters such as acetic acid rate, duration of fumigation, and type of container used during fumigation were assessed as well as apple quality, storage disorders and vinegar aroma.

2. Materials and methods

All apples used in this study were harvested at commercial maturity from an orchard located at the Pacific Agri-Food Research Centre (PARC), Summerland, British Columbia, Canada, and placed in (1.0 ± 0.2) °C cold storage until needed. All fumigation

trials were done at least three times with laboratory grade glacial acetic acid (AA) in a 1 m^3 fumigation chamber as previously described [17] at 10 °C, on fruit that had been stored at 10 °C for at least 16 h prior to fumigation.

2.1. Apple sanitation study

'Gala', 'McIntosh', 'Delicious' (Golden and Red), 'Jonagold', and 'Spartan' apples stored for (7 to 8) months in air were used in this study. Sixteen kg of each apple cultivar were placed in small wooden bins (27.5 cm high by 48 cm wide by 61 cm long), 1/20th the size of standard apple bins. Three of these model bins with each bin filled with 10 kg of apples of a particular cultivar were fumigated with 10 µL glacial acetic acid × L^{-1} vapor. After fumigation for either (1, 1.5, 2 or 24) h, the chamber was opened and allowed to vent for at least 30 min.

Both fumigated and control fruit were subsampled by taking 30 apples from each model bin replicate, punctured at three locations on each fruit with a 5 cm finishing nail that protruded 6 mm from a cork stopper. The protruding end of the nail was kept sterile by dipping in 95% ethanol and flaming after wounding 10 apples. The apples were placed at (20 ± 1) °C for 1 week and the number of punctures that decayed were recorded. The experiment was repeated three times.

Analysis of variance was applied to the arcsine-transformed cultivar data with the General Linear Model (GLM) procedure (SAS Institute, Cary, NC) and following a significant F test ($P \geq 0.05$) means were separated with Duncan's Multiple Range Test ($P = 0.05$).

2.2. Bagged apple study

'Spartan' apples were segregated into lots based upon treatment, inoculation with *Penicillium expansum*, and whether punctured with a sterile nail. The apples were inoculated by misting with a 6.2×10^4 colony-forming units (CFU) × mL^{-1} conidial suspension of *P. expansum* counted with a haemacytometer, and then punctured as described above. The fruit were fumigated with 7.5 µL glacial acetic acid × L^{-1} as described previously. After venting the remaining acetic acid vapor, the fruit was placed in plastic packing bags with ventilation holes (1.5 kg fruit). The bagged apples were stored at (5.0 ± 0.2) °C for 30 d followed by 7 d at (20 ± 1) °C, when the numbers of decayed fruit were recorded. Each treatment factor was replicated four times. The data were analyzed as a triple factorial experiment with the GLM procedure of SAS (SAS Institute, Cary, NC).

In a second trial, 'McIntosh' and 'Delicious (Red)' apples were used. Acetic acid fumigation (7.5 µL × L^{-1}) was compared with the standard postharvest fungicide treatment of thiabendazole. The apples were fumigated or dipped for 1 min in a 450 µL thiabendazole × L^{-1} solution (Merteck 45% flowable, Montreal, Quebec, Canada). Following treatment, the apples were bagged and stored at (20 ± 1) °C for 21 d, and the number of decayed apples were recorded. Analysis of variance was applied to the arcsine-transformed data with the GLM procedure (SAS Institute, Cary, NC) and means were separated with Duncan's Multiple Range Test ($P = 0.05$).

2.3. Long term apple storage study

The effect of AA fumigation on stored apples was studied in three trials. In a first trial, 10 kg of Red 'Delicious', Golden 'Delicious' and 'Spartan' apples were placed into plastic boxes, inoculated by misting with 1×10^6 CFU *P. expansum* × mL^{-1}, allowed to dry and dipped for 0.5 min in 450 µL thiabendazole × L^{-1} or fumigated with 7.5 µL acetic acid × L^{-1}. Apples were stored at (1 ± 0.2) °C for 3 months, removed from storage and a subsample of 15 fruits from each replicate was punctured as described above, placed at (20 ± 1) °C for 1 week, and the number of decayed punctures were recorded. Analysis of variance was applied to the arcsine-transformed data with the GLM procedure (SAS Institute, Cary, NC) and means were separated with Duncan's Multiple Range Test ($P = 0.05$).

In a second experiment, 'McIntosh' apples received multiple fumigations to test

their effect on apple quality. Apples were fumigated from one to eight times starting immediately after the fruit was placed in cold storage at (1 ± 0.2) °C for 111 d. Physiological disorders such as lenticel injury and scald were visually assessed on fruit from storage after a ripening period of 7 d at 20 °C.

A third trial was conducted in two parts, with apple quality and aroma evaluated in the first part, and decay in the second part.

In the quality and aroma trial, 14 kg of 'Gala', 'Jonagold' and 'Spartan' apples were placed in model bins, interlocking plastic apple boxes (25 cm × 30 cm × 45 cm) and standard wooden apple boxes (30 cm × 30 cm × 45 cm). Fruit stacked three boxes high were fumigated for 24 h with 9.0 µL AA × L^{-1}. After venting for 1 h to 2 h, apples were stored at (1 ± 0.2) °C for 90 d. Upon removal from storage, subsamples of ten and six 'Gala' apples were taken for quality and aroma tests, respectively. Lenticel burning was visually assessed. Soluble solids and titratable acidity were determined by juicing each apple and measuring percent soluble solids with an Abbé refractometer (AO instruments, Buffalo, NY), and titratable acidity with a Brinkmann Titroprocessor ensemble (Metrohm, Herisau, Switzerland). Analysis of variance was applied to the arcsine-transformed data with the GLM procedure (SAS Institute, Cary, NC) and means were separated with Duncan's Multiple Range Test (P = 0.05).

The aroma of acetic acid can be described as a vinegar aroma. Therefore, vinegar aroma was evaluated by a panel of 16 subjects (employees of Agriculture and Agri-Food Canada). They evaluated both whole and sliced apples from the six treatments from storage, and a freshly fumigated treatment done 16 h prior to sensory evaluation. A random sample of six apples was obtained from treated and nontreated 'Gala' apples. For the whole apples, four apples were placed in 2 L plastic buckets with removable plastic lids. For the sliced apples, two apple slices from each treatment were placed in 250 mL glasses and covered with plastic lids. Samples were left at room temperature overnight and assessed the following morning with half the panelists evaluating the whole apples first and half evaluating the sliced apples first.

Subjects lifted the lids of the sample containers and sniffed the samples in random order indicating the presence or absence of perceived acetic acid/vinegar aroma. Responses were tabulated and recorded and significance of responses analyzed statistically by the method of Csima and Reid [20].

The second part of the trial evaluated decay. Apples were stored for 4 months at (1.0 ± 0.2) °C before they were used. Fruit in plastic and wooden boxes were fumigated with 7.0 µL AA × L^{-1} while the larger model bins were fumigated with 8.0 µL AA × L^{-1} before and after storage for 90 d. Decay was evaluated by recording the total number of decayed fruit after storage. Fifteen fruit were subsampled after the final AA fumigation. The number of decayed punctures was recorded after 6 d at (20 ± 1) °C. The arcsine-transformed data were analyzed as a double factorial experiment with treatment and boxes as main effects with the GLM procedure of SAS (SAS Institute, Cary, NC) and means were separated with Duncan's Multiple Range Test (P = 0.05).

3. Results

3.1. Apple sanitation

Fumigation with 10 µL AA × L^{-1} reduced the percentage of decayed punctures in 'Gala', 'McIntosh', Golden 'Delicious', Red 'Delicious', and 'Spartan' apples after (7 to 8) months of storage at 1 °C (*table I*). Extending the fumigation duration from (1 to 24) h did not improve decay control significantly.

3.2. Bagged apples

Fumigation with 7.5 µL AA × L^{-1} significantly reduced the number of 'Spartan' apples that decayed in bags stored at 5 °C for 30 d (*table II*). In a shelf life trial with

McIntosh' apples where thiabendazole postharvest treatment was compared with AA fumigation, only AA fumigation was effective in reducing decay (*table III*).

3.3. Long term apple storage

Fumigation with 7.5 µL AA × L^{-1} reduced the percent of 'Spartan' apples that decayed

Table I.
Effect of acetic acid fumigation in preventing decay in wounded apples.

Apple cultivar	Treatment[1]	Treatment duration (h)	% decay
Gala	Control	0.0	80.9 a
	Acetic acid	2.0	1.1 b
McIntosh	Control	0.0	77.8 a
	Acetic acid	1.0	10.3 b
Golden Delicious	Control	0.0	61.5 a
	Acetic acid	1.5	6.2 b
	Acetic acid	24.0	2.3 b
Jonagold	Control	0.0	48.5 a
	Acetic acid	1.5	2.9 b
	Acetic acid	2.0	5.8 b
Red Delicious	Control	0.0	54.8 a
	Acetic acid	1.0	1.7 b
	Acetic acid	2.0	1.1 b
	Acetic acid	24.0	1.7 b
Spartan	Control	0.0	69.9 a
	Acetic acid	1.0	5.2 b
	Acetic acid	2.0	1.7 b
	Acetic acid	24.0	4.4 b

[1] Fruit stored for (7 to 8) months in air at 1 °C were placed in small wooden bins, fumigated with 10 µL acetic acid × L^{-1} for 1 h to 24 h, subsampled (30 apples per replicate), wounded, placed at 20 °C for 1 week, and the number of decayed punctures recorded. Each cultivar trial was repeated three times.
Means within each cultivar followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P = 0.05$).

Table II.
Effect of acetic acid fumigation on bagged 'Spartan' apples.

Treatment[1]	% decayed fruit in		
	untreated fruit	inoculated fruit[2]	punctured fruit
Control	9.4	17.0	50.9
Acetic acid	4.2**	14.6****	35.7****

[1] 'Spartan' apples were fumigated with 7.5 µL acetic acid × L^{-1}, stored in plastic apple bags (1.5 kg) at 5 °C for 30 d, 20 °C for 7 d, and the number of decayed fruit recorded.
[2] Apples were inoculated by misting with a 6.2×10^4 colony-forming units (CFU) × mL^{-1} conidial suspension of *Penicillium expansum*.
, **: significant at $P \leq 0.01$ and $P \leq 0.0001$, respectively.

Table III.
Decayed fruit of two apple cultivars treated with acetic acid or thiabendazole.

Treatment[1]	Decayed fruit (%) in	
	McIntosh	Red Delicious
Control	38.8 a	8.1
Acetic acid	4.1 b	5.0
Thiabendazole	59.9 a	5.1

[1] Apples were stored for 5 months at 1 °C, treated with 7.5 µL acetic acid × L^{-1} or 450 µg thiabendazole × L^{-1}, placed at 20 °C for 21 d, and the number of decayed apples recorded.
Means followed by the same letter in a column are not significantly different according to Duncan's Multiple Range Test ($P = 0.05$).

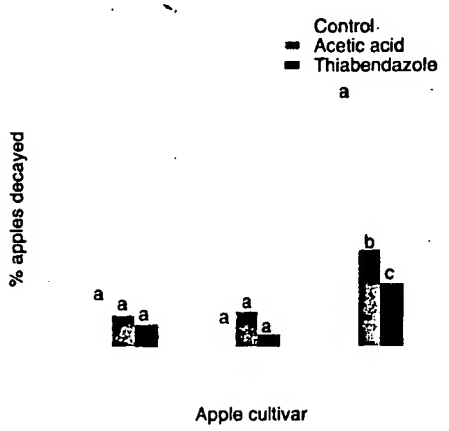

Figure 1.
Percent of apples decayed that were treated with 7.5 µL glacial acetic acid × L^{-1} or dipped for 0.5 min in 450 µg thiabendazole × L^{-1} and stored for 3 months at 1 °C. Means were separated with the Duncan's Multiple Range Test ($P = 0.05$).

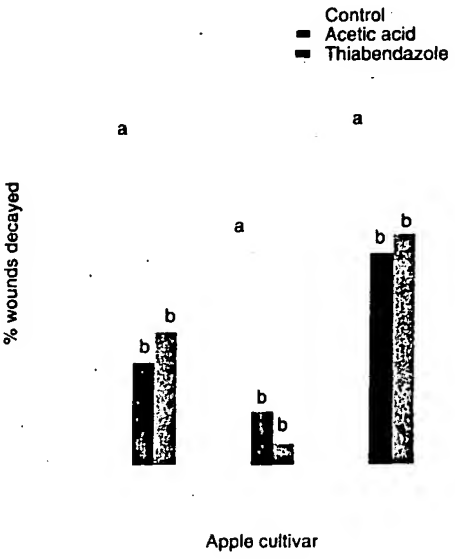

Figure 2.
Percent wounds (nail punctures) that decayed in apples from storage for 102 d at 1 °C that were treated by acetic acid fumigation (7.5 µL × L^{-1}) or dipping for 0.5 min in 450 µg thiabendazole × L^{-1}. Prior to storage, the apples had been inoculated with *Penicillium expansum*, and treated with 7.5 µL glacial acetic acid × L^{-1} or dipped for 1 min in a 450 µg thiabendazole × L^{-1} solution. Means were separated with the Duncan's Multiple Range Test ($P = 0.05$).

over storage for 3 months at 1 °C and 1 week at 20 °C (*figure 1*). If the apples were punctured, AA fumigation reduced the percent of decay in Golden 'Delicious', Red 'Delicious' and 'Spartan' apples and was just as affective as thiabendazole (*figure 2*).

Damage to the fruit skin caused by multiple AA fumigations was studied in 'McIntosh' apples stored for 111 d. Lenticel damage increased tremendously with four to eight fumigations (*table IV*). Scald, another important disorder of stored apples, was not affected by AA fumigation.

The effect of one fumigation with 9.0 µL AA × L^{-1} was studied in more detail in subsequent experiments. After 90 d storage, significant lenticel damage was found in AA fumigated 'Gala' and 'Spartan' apples (*table V*). AA fumigation did not affect percent soluble solids or titratable acidity. The type of container used for AA fumigation influenced the percent of fruit with lenticel damage (*table VI*), with the plastic box corresponding to higher lenticel damage for 'Gala' apples. The model wooden bin had the lowest levels of lenticel damage for 'Spartan' apples.

'Gala' apples from the above trial had no detectable acetic/vinegar aroma in treated whole apples that had been stored for 90 d, although all the subjects detected vinegar aroma in 'freshly' fumigated apples. The results were almost the same for sliced apples except that only 19% of the subjects could detect the vinegar aroma in the 'freshly' fumigated apple slices.

Fumigation of naturally contaminated fruit with 7.0 µL AA × L^{-1} reduced decay in 'Jonagold' apples by approximately one half compared with the untreated control after the fruit had been fumigated and stored for 90 d (*table VII*). The use of AA fumigation immediately before injuring fruit after storage reduced the number of punctures that decayed from 32% to 6% in 'Gala' and 32% to 12% in 'Jonagold'. Analysis of the data indicated that there was a significant container effect on decay in 'Gala' and 'Jonagold' apples (*table VII*). Fewer decayed punctures occurred in 'Jonagold' apples that were fumigated in the model bin or wooden apple box (*table VIII*). In 'Gala', the lowest percentage of decayed punctures was in

fruit fumigated in the model bin. Container type had no effect on decay of 'Spartan' apples.

4. Discussion

AA fumigation shows great potential for reducing postharvest decay on apples stored under conditions that are similar to those used in commercial storage. The treatment is very effective when it is used to sterilize the surface of the fruit preventing decay. Results showed that AA fumigation reduced decay in apple punctures to insignificant levels in 'Gala', 'Jonagold', Red 'Delicious', and 'Spartan' apple cultivars (*table I*). The higher-than-usual decay level

Table IV.
Effect of multiple acetic acid fumigations (7.5 µL × L^{-1}) on 'McIntosh' apple quality after 111 d of storage at 1 °C.

Timing	Frequency	% lenticel damage	% scald
None	0	5.3 b	91.8
Prior to storage	1	5.3 b	67.5
Once per month	4	39.9 a	96.5
Twice per month	8	45.2 a	96.5

Means in columns followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P = 0.05$).

Table V.
Effect of acetic acid fumigation (9.0 µL × L^{-1}) on quality of apples stored for 90 d at 1 °C in three container types.

Apple cultivar	Treatment	% lenticel damage	% soluble solids	Titratable acidity
Gala	Control	8.3	12.87	6.64
	Acetic acid	25.8	12.85	6.90
Significance:				
Treatment		**	ns	ns
Container		**	ns	ns
Treatment × container		**	*	ns
Jonagold	Control	3.2	13.27	8.28
	Acetic acid	45.6	13.07	8.29
Significance:				
Treatment		ns	ns	ns
Container		ns	ns	ns
Treatment × container		ns	ns	ns
Spartan	Control	3.2	13.40	8.18
	Acetic acid	28.9	13.48	7.80
Significance:				
Treatment		***	ns	ns
Container		**	ns	ns
Treatment × container		*	ns	ns

*, **, ***, ns: significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, or not significant, respectively.

of 10% in AA fumigated 'McIntosh' apples likely was the result of a heavy spore load on the fruit. Sholberg and Gaunce [13] showed that, as the inoculum concentration of *P. expansum* conidia increased, the degree of control decreased if the AA concentration remained constant.

In some trials, apples were inoculated with *P. expansum* and artificially wounded by puncturing with a nail to insure decay. These trials showed that AA fumigation was just as effective as thiabendazole in preventing decay on fruit inoculated with *P. expansum*. In one particular trial,

Table VI.
Effect of container type on percent lenticel damage of apples stored for 90 d at 1 °C.

Container type	% lenticel damage in apple cultivar		
	Gala	Jonagold	Spartan
Wooden apple box	10.7 a	3.6 a	19.6 b
Model wooden bin	5.1 a	7.2 a	3.6 a
Plastic apple box	62.6 b	24.3 a	20.5 b

Means within columns followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P = 0.05$).

Table VII.
Effect of acetic acid fumigation (7.0 µL × L^{-1}) before and after storage on decay of apples stored for 90 d at 1 °C in three container types.

Apple cultivar	Treatment	% decayed apples	% decayed wounds
Gala	Control	4.2	32.3
	Acetic acid	2.7	5.8
Significance:			
Treatment		ns	***
Container		ns	*
Treatment × container		ns	ns
Jonagold	Control	21.1	31.9
	Acetic acid	11.1	12.4
Significance:			
Treatment		*	*
Container		ns	*
Treatment × container		*	ns
Spartan	Control	34.2	62.4
	Acetic acid	28.0	40.9
Significance:			
Treatment		ns	ns
Container		ns	ns
Treatment × container		ns	ns

Apples were stored for 4 months in air at 1 °C before the start of the trial.
*, ***, ns: significant at $P \leq 0.1$, $P \leq 0.01$, or not significant, respectively.

not show any sign of corrosion. Acetic acid will eventually corrode steel and copper parts that are not protected by an acid-resistant coating.

Use of AA fumigation is a viable option or complement to the use of other postharvest treatments such as thiabendazole or sodium hypochlorite for control of postharvest decay in stored apples. AA fumigation is suited for use on apples destined for processing and/or where an organic alternative to synthetic fungicides is required. AA fumigation meets these requirements because processing fruit usually have a high potential for decay, acetic acid is relatively cheap, and the fruit may be fumigated without removal from the bins. Another important consideration for using AA fumigation is the concern with patulin in processed juice products such as apple juice [25]. Timely use of AA fumigation will reduce the incidence of *Penicillium* spp. infection to minimal levels and thus curtail any production of patulin by the fungus.

In conclusion, AA fumigation before and after storage reduces decay and the potential for decay in wounded fruit. If used at rates previously determined by careful experimentation, in consideration of cultivar, temperature, volume of fruit, and container type, AA will not damage fruit lenticels or have any deleterious effect on quality or aroma.

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References

[1] Snowdon A.L., A color atlas of post-harvest diseases and disorders of fruits and vegetables, Vol. 1: General introduction & Fruits, CRC Press, Inc., Boca Raton, Florida, USA, 1990.

[2] Sholberg P., Postharvest handling of pome fruits, soft fruits, and grapes. Agriculture Canada, Publication 1768E, Ottawa, Canada, 2000, Available as an electronic publication http://res2agr.ca/parc-crapac/english/3electronic_publications/phhandbook/default.htm

[3] Archibald S.O., Winter C.K., Pesticides in our food. Assessing the risks, in: Winter C.K., Seiber J.N., Nuckton C.F. (Eds), Chemicals in the human food chain, Van Nostrand Reinhold, New York, USA, 1990.

[4] Sholberg P.L., Haag P.D., Incidence of postharvest pathogens of stored apples in British Columbia, Can. J. Plant Pathol. 18 (1996) 81–85.

[5] Rosenberger D.A., Blue mold, in: Jones A.L., Aldwinckle H.S. (Eds), Compendium of apple and pear diseases, APS Press, St. Paul, USA, 1990.

[6] Eckert J.W., Dynamics of benzimidazole-resistant Penicillia in the development of postharvest decays of citrus and pome fruits, in: Delp C.J. (Ed.), Fungicide resistance in North America, APS Press, St. Paul, USA, 1988.

[7] Bond E.J., Chemical control of stored grain insects and mites, in: Sinha R.N., Muir W.E (Eds), Grain storage: part of a system, The AVI Publishing Co., Inc., Wesport, CT, USA, 1973.

[8] Eckert J.W., Ogawa J.M., The chemical control of postharvest diseases: deciduous fruits, berries, vegetables and root/tuber crops, Ann. Rev. Phytopathol. 26 (1988) 433–469.

[9] Stadelbacher G.J., Prasad K., Postharvest decay control of apple by acetaldehyde vapor, J. Am. Soc. Hortic. Sci. 99 (1974) 364–368.

[10] Mattheis J.P., Roberts R.G., Fumigation of sweet cherry (*Prunus avium* 'Bing') fruit with low molecular weight aldehydes for postharvest decay control, Plant Dis. 77 (1993) 810–814.

[11] Yuen C.M.C., Paton J.E., Hanawati R., Shen L.Q., Effects of ethanol, acetaldehyde and ethyl formate vapour on growth of *Penicillium italicum* and *P. digitatum* on oranges, J. Hortic. Sci. 70 (1995) 81–84.

[12] Agreda V.H., Zoeller J.R., Acetic acid and its derivatives, Marcel Dekker, Inc., New York, USA, 1993.

[13] Sholberg P.L., Gaunce A.P., Fumigation of fruit with acetic acid to prevent postharvest decay, HortScience 30 (1995) 1271–1275.

Table VIII.
Effect of container type on the percent of wounds that decayed in acetic acid fumigated (7.0 µL × L^{-1}) apples stored for 90 d at 1 °C.

Container type	% decayed wounds in apple cultivar		
	Gala	Jonagold	Spartan
Wooden apple box	16.8 ab	14.3 b	56.8 a
Model wooden bin	9.6 b	12.0 b	46.6 a
Plastic apple box	26.0 a	41.3 a	51.6 a

Means within columns followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P = 0.05$).

AA fumigation was much more effective than thiabendazole on 'McIntosh' apples (*table III*). The reason that thiabendazole did not control decay could have been fungicide resistance as reported in New York [21], Washington [22] and Oregon [23] and in British Columbia, Canada [4].

AA fumigation was effective in reducing decay in relatively large volumes of fruit stored for long periods of time. The source of inoculum did not change the effectiveness of AA fumigation. For example, decay was reduced in both 'Spartan' apples inoculated with *P. expansum* conidia and 'Jonagold' apples naturally contaminated by *P. expansum*.

Four or more fumigations with AA caused lenticel damage in 'McIntosh' apples (*table IV*). Previous research had indicated that phytotoxicity caused by AA vapor could be an important problem. For example, Sholberg [16] had shown that AA fumigation could cause pitting of Bing cherries and Sholberg and Gaunce [14] showed that AA fumigation would cause browning on peaches if the AA concentration was not carefully controlled. In these apple trials, no AA fumigations appeared to be safe if AA were below 8.0 µL AA × L^{-1}. However, for numerous AA trials on apples, it has become apparent that decay control and lenticel burning and blackening vary with frequency of fumigation treatment, AA concentration, fruit quantity, container type, and apple cultivar. Previous trials in small chambers demonstrated that humidity, temperature, and duration of AA fumigation affect decay control [11].

AA fumigation requires constant monitoring if lenticel burning is to be avoided. Early attempts at monitoring AA concentration during fumigation relied on Kitagawa precision detector tubes (Matheson Safety Products, East Rutherford, NJ) [17]. A sample of acetic acid vapor was drawn into the tube reacting with sodium hydroxide and resulting in a proportional color change that was read in parts per million. This method, although useful for indicating the presence of AA vapor, was found to be costly and inaccurate for AA fumigation. Recent attempts at monitoring AA fumigation have relied on the use of a gas chromatograph and/or solid state sensors [24].

A question that must be answered before acetic acid can be used commercially is if it can be detected in fruit by taste or smell. Vinegar aroma was not detected in AA fumigated 'Gala' apples, suggesting that lenticel burning is the only major quality concern with AA fumigation of apples.

In this study, corrosion of metal by AA fumigation was minimal because use rates were very low and most fumigations were restricted to short durations before and after the fruit had been stored. There was no indication of damage to metal bin parts such as nails; however, after 50 or more fumigations damage began to show on the metal fan impeller used for air circulation in the 1 m^3 chamber. The rest of the chamber that was lined with aluminum foil did

[14] Sholberg P.L., Gaunce A.P., Fumigation of stone fruit with acetic acid to control postharvest decay, Crop Prot. 15 (1996) 681–686.

[15] Chu C.L., Liu W.T., Zhou T., Tsao R., Control of postharvest gray mold rot of modified atmosphere packaged sweet cherries by fumigation with thymol and acetic acid, Can. J. Plant Sci. 79 (1999) 685–689.

[16] Sholberg P.L., Fumigation of fruit with short-chain organic acids to reduce the potential of postharvest decay, Plant Dis. 80 (1998) 689–693.

[17] Sholberg P.L., Reynolds A.G., Gaunce A.P., Fumigation of table grapes with acetic acid to prevent postharvest decay, Plant Dis. 80 (1996) 1425–1428.

[18] Moyls A.L., Sholberg P.L., Gaunce A.P., Modified-atmosphere packaging of grapes and strawberries fumigated with acetic acid, HortScience 31 (1996) 414–416.

[19] Sholberg P.L., Delaquis P.J., Moyls A.L., Use of acetic acid fumigation to reduce the potential for decay in harvested crops, Recent Res. Dev. Plant Pathol. 2 (1998) 31–42.

[20] Csima A., Reid D.B.W., Table for testing the significance of the difference between proportions, University of Toronto, Department of Epidemiology and Biometrics, Toronto, Canada, 1961.

[21] Rosenberger D.A., Wickow D.T., Korjagin V.A., Rondinaro S.M., Pathogenicity and benzimidazole resistance in *Penicillium* species recovered from flotation tanks in apple packinghouses, Plant Dis. 75 (1991) 712–715.

[22] Spotts R.A., Cervantes L.A., Populations, pathogenicity, and benomyl resistance of *Botrytis* spp., *Penicillium* spp., and *Mucor piriformis* in packinghouses, Plant Dis. 70 (1986) 106–108.

[23] Bertrand P.F., Saulie-Carter J.L., The occurrence of benomyl-tolerant strains of *Penicillium expansum* and *Botrytis cinerea* in the Mid-Columbia region of Oregon and Washington, Plant Dis. Rep. 62 (1978) 302–305.

[24] Sholberg P., Shephard T., Moyls L., A novel detection system for continuous monitoring of acetic acid vapour concentrations for decay control, (Abstract) Can. Inst. Food Sci. Tech. Annu. Meet., Kelowna, British Columbia, Canada, 1999.

[25] Moss M.O., The occurrence and significance of mould toxins (mycotoxins) in food, Food Sci. Technol. Today 9 (1995) 35–38.

Fumigación con vapor de ácido acético para controlar el deterioro de manzanas almacenadas.

Resumen — Introducción. Las manzanas están potencialmente expuestas a la marchitez azul del manzano causada por *Penicillium expansum* si se almacenan a 1 °C durante 3 meses o más y si sufren daños durante la manipulación. Los resultados de ensayos efectuados con manzanas contaminadas por conidios de *P. expansum* y fumigadas en un local reducido con vapor de ácido acético (AA) mostraron que los frutos esterilizados podían deteriorarse menos sin que su calidad se viera alterada. El objetivo de este trabajo ha sido determinar si era posible proteger cantidades mayores de manzanas contra dichos deterioros tras almacenamiento y/o después de las heridas tratándolas con vapor de AA. Se trataba, asimismo, de determinar si la fumigación afectaba a la calidad y al aroma de las manzanas tratadas. **Material y métodos**. Se escogieron manzanas de diferentes cultivares en la fase de madurez comercial y se utilizaron en pruebas de fumigación con AA. Se fumigaron manzanas, artificial o naturalmente contaminadas con conidios de *P. expansum*, con vapor de AA en una cámara de 1 m^3, estanca al gas, a 20 °C durante 1 h a 24 h o sumergidas en una solución de tiabendazol a 450 $\mu g \times L^{-1}$. Los frutos fumigados fueron dañados y, posteriormente, se evaluó su deterioro tras una semana a 20 °C o bien almacenados a 1 °C durante 3 meses o más y, seguidamente, se evaluó su estado. Se estimó, entonces, la calidad de las manzanas. **Resultados**. Las manzanas naturalmente contaminadas con *Penicillium* spp., almacenadas al aire a 1 °C y tratadas con vapor de AA sufrieron la mitad de daños que los frutos testigos. En otro experimento, la fumigación con AA fue tan eficaz como el tiabendazol para reducir la marchitez. La fumigación con AA redujo el deterioro de los frutos tras almacenamiento durante 3 meses y un segundo tratamiento con